Peer Review
Experimental Toxicology Division
N H E E R L

EMERGING ISSUES

The Developmental Immunotoxicity of Endocrine-disrupting Chemicals

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Introduction

Endocrine Disrupting Chemicals – also disrupt immunity

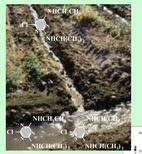
- Toxicant exposure during development may produce persistent adverse effects—suggesting increased sensitivity of immature animals—within multiple systems:
  - •Endocrine
  - •Nervous
  - •Immune
- Multi-system effects suggest possible shared modes of action.
- Immune system is strongly influenced by endocrine and central nervous systems.

Objectives

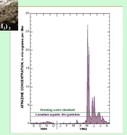
- Broad Objective: To determine whether developmental exposure to pesticides that are known endocrine disrupting chemicals (EDCs) causes persistent immunotoxicity, and to identify endocrine or CNS factors that mediate toxicity.

Atrazine (ATR)

ATR is a broadleaf herbicide used in production of corn, sorghum and sugar cane
#1 pesticide in the world by area with about 75 million lbs. spread annually in US alone



- •ATR has a ½-life of ≥2 years and moderate solubility
- •ATR is the most commonly detected pesticide in ground and surface water in US
- •Maximum Concentration Limit (MCL) in water of 3 ppb is often exceeded in spring-associated spikes



ATR Toxicity

- •ATR has limited immunotoxicity in adults
- •Endocrine toxicity of ATR
  - •Decreases prolactin (PRL) in adult and milk
    - •Low PRL in milk → Leads to low dopamine in TIDA (PRL-secretion-regulator) neuron in offspring
    - •Results in abnormally high PRL secretion as adult
  - •Decreases thyroid hormones (THs) at high doses

Methods

Project 1. Characterization of ATR as a developmental immunotoxicant

A. Treatment of dams during pregnancy and lactation

- Vehicle Control
- Atrazine (35mg/kg/day or 10x LOAEL)
- Decrease Prolactin (using Bromocryptine) to model ATR's block of PRL
- Decrease Thyroid Hormones (using Propylthiouracil) to model ATR's block of THs

ATR Exposure or Hormone Block

Gestation | Lactation | Immune Maturation

GD10 PND22 PND50+

B. Test immune function

- Delayed-type hypersensitivity
- Antibody response to SRBC (sheep red blood cells)
- NK function
- Phagocytosis

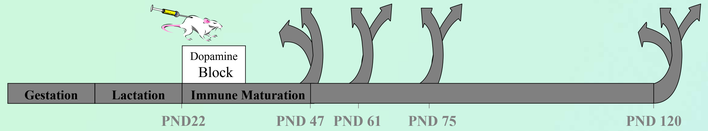
Project 2. Test autoimmunity as a mode of action for prostate inflammation associated with ATR-induced dysregulation of PRL homeostasis

A. Treatment of offspring

- Vehicle Control
- Dopamine (Pimozide)
  - (20mg/kg)
  - to model ATR's block of Dopamine from decreased PRL in milk resulting in INCREASED PRL as juvenile and then Prostatitis

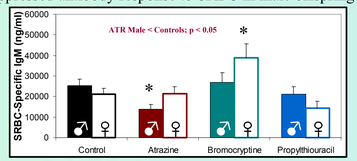
B. Test for autoimmunity associated with prostatitis

- Antibody response to SRBC
- Antibody response to prostate antigens)
- Lymphoproliferative response to prostate antigen
- Inflammatory cytokines (IL2, IL6, IL10, IFNγ) (TNFα, MIP-1α, and ICAM-1)

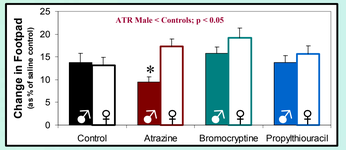


Results

ATR suppressed antibody response to SRBC in male offspring only



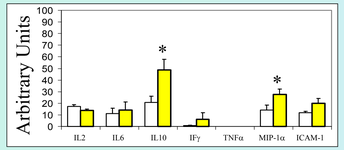
ATR suppressed delayed-type hypersensitivity response in male offspring only



No signs of Autoimmunity in dopamine suppressed model of ATR-exposed animals
Prostate antigens were not recognized by the immune system of experimental pups
No change in antibody production

Increased cytokine message prior to visual detection of prostatitis

Pimozide stimulated proinflammatory cytokines



Conclusions and Impact

Conclusions

Project 1

•ATR is not immunotoxic in adult rats. Thus, ATR joins the list of xenobiotics for which the developing immune system is more sensitive than the mature system.

•And, while gender sensitivity suggests a hormonal mode of action, studies with hormone antagonists suggest that neither PRL nor THs are the proximate mechanisms of ATR immunotoxicity.

Project 2

•Classical autoimmunity does not appear to be the cause of PRL-associated prostatitis.

•On the other hand, message for proinflammatory cytokines and an adhesion molecule, associated with immune system activation were upregulated in lobes of the pimozide group, indicating a treatment-related change in at least innate immune responsiveness.

Impact

There is currently a great deal of controversy over the potential health risk posed by the presence of ATR in surface and ground water. The ATR studies will provide insight into the potency of ATR as a developmental immunotoxicant, vs. its potency as a reproductive EDC, and shed light on similar modes of toxicity in the immune and endocrine systems.

Future studies on the role of GnRH as a gender-specific determinant in ATR-induced immunotoxicity will increase our understanding of how EDCs may affect the development and maturation of the immune system. These studies will also provide us with a greater understanding of the complex relationship between development and maturation of the immune and endocrine systems.

Nonbacterial prostatitis affects a significant number of men worldwide, and has been reported more frequently in pesticide applicators. Although autoimmunity has an as yet undetermined role in the etiology of the disease, this project will provide insight into the role that EDCs may play in prostate inflammation.

Our work will aid the risk assessment process by determining possible long term immune system effects of exposure to EDCs at the organism level, and identify potential consequences of exposure to EDCs during development of the immune and endocrine systems.

Future Directions

Project 1

Paracrine gonadotropin releasing hormone (GnRH) circuits within the immune system may contribute to gender differences in immune function and autoimmune disease. ATR toxicity includes suppression of the pulsatile release of GnRH from the hypothalamus and may also disrupt paracrine GnRH regulation within the immune system. Clarifying the relationship between ATR exposure, GnRH activity, and antigen-driven immune responses may identify how ATR causes immunotoxicity and contribute to our understanding of gender-influenced immunotoxicity in general.

Project 2

Characterization of the cell types present in inflamed prostate, their state of activation, as well as cytokine message and protein expression in the pre-inflammatory phase of the disease will help to define the nature of the inflammatory response. We will continue to pursue our current hypotheses that architectural or physiological changes occur in the prostate that ultimately leads to inflammation, that is augmented by the immunostimulating properties of PRL.

*photos from "whole-crop.com" and Gloria-Leigh Logan with permission

SOLVING AGENCY PROBLEMS